compounds for anxiety-modulating activity. Prior to this discovery of the inventors, PKCε was not known to be associated with anxiety.

The relationship between PKCε and anxiety was demonstrated in the behavior of a mutant mouse strain, created by the present inventors, that is homozygous null for PKCε. These PKCε$^{-/-}$ mice were indistinguishable from their wild type littermates in characteristics such as body weight, eating and drinking (see specification at page 55, lines 6-8), and demonstrated normal spontaneous locomotor behavior and habituation to a novel environment (page 55, lines 8-10). However, in at least two tests that are commonly used to assess animal anxiety levels, the open-field test and the elevated plus maze, the PKCε$^{-/-}$ mice differed in certain key behaviors from their wildtype littermates which suggested that the PKCε$^{-/-}$ mice had reduced anxiety levels (specification at page 55, lines 13-24). These findings alone suggest a role for PKCε in anxiety.

To add to the evidence for the relationship between PKCε and anxiety, Applicant has demonstrated a link between PKCε and $GABA_A$ receptor. $GABA_A$ receptor agonists are well known to have anxiolytic properties. As described in the specification at page 5, lines 11-17, benzodiazepines, a class of drugs commonly used to reduce anxiety, bind to and allosterically potentiate the actions of GABA at $GABA_A$ receptors. Applicant has shown that PKCε$^{-/-}$ mice are more sensitive to the sedative effects of diazepam, a benzodiazepine, than are wild type mice, as measured by loss of righting reflex following administration of diazepam (see specification, Example 6, at pages 59-60), and that $GABA_A$ receptors from the frontal cortex of PKCε$^{-/-}$ mice are more sensitive to allosteric modulation by benzodiazepines than are $GABA_A$ receptors from the frontal cortex of wild type mice, as measured by enhancement of muscimol-stimulated uptake of Cl^- in the presence of flunitrazepam, another benzodiazepine (Example 7, pages 60-61, particularly at page 61, lines 14-20). A similar enhancement of muscimol-stimulated uptake of Cl^- was seen with $GABA_A$ receptors from wild type mice that had been treated with the selective PKCε inhibitor, εV1-2, (thus making these wild type samples effectively PKCε$^-$)(specification at page 61, line 21 through page 62, line 11). These experiments strongly support the conclusion that it is the absence of PKCε in the knockout mice that is responsible for the enhanced sensitivity to allosteric modulators

(both exogenous and endogenous) of the $GABA_A$ receptors seen in those mutant mice. Given the prominent role of $GABA_A$ receptors in modulation of anxiety, this enhanced sensitivity contributes to a lower anxiety level in the mutant mice.

The inventors' discovery of the relationship of PKCε and anxiety makes possible rapid high-throughput screening assays using modulation (that is, inhibition or enhancement) of PKCε activity to identify candidate anxiolytic or anxiogenic compounds Such candidate compounds may be further tested in well known animal test systems for modulation of anxiety. Claim 32 is directed to the method for identifying compounds that modulate anxiety by screening for the compound's effect on the activity of PKCε. Claim 32 has now been limited to screening for modulation of the activity of PKCε in other than an animal system. Claim 32 now encompasses screening assays performed, for example, in isolated cells (e.g., in a transformed cell culture), in a cell lysate, or with a purified PKCε protein. Claim 35 is directed to the embodiment of the method for identifying compounds that modulate anxiety that combines the PKCε activity screening method, as in Claim 32, with an *in vivo* screening method for determination of the test compound's ultimate effect on anxiety in an animal. Thus, in the method of claim 35, compounds that are selected as modulators of PKCε activity are further screened for their effects on anxiety in a test animal. In another embodiment of the method of the invention, compounds that have already been identified as modulators of PKCε activity are provided and screened *in vivo* in a test animal to determine their effects on anxiety in the animal. Such an embodiment is encompassed by claim 10.

The Amendments

Claims 1-9, 11-28 and 40-53, which were previously withdrawn as directed to a non-elected invention, have now been cancelled. Claim 10 has been amended to clarify that the test compound is administered to a test animal and a step of determining the modulation of anxiety in the test animal has been added. Claim 31 has been amended to clarify that the recited Markush group members relate to symptoms of anxiety in test animals and that reduction of anxiety in the test animal is determined by reduction in one or more of the recited symptoms. Claim 32 has been amended by replacing the "exposing" step with a "measuring" step similar to that in claim 35 and by adding the

limitation that the measuring is performed in other than an animal. Claim 35 has also been amended to limit the "measuring" of PKCε activity to one performed in other than an animal. Claims 33 and 36 have been amended to limit the measuring of claims 32 and 35 to one performed in an *isolated* cell or cell lysate. Minor amendments have been made to claims 38 and 39.

New claims 54 and 55 have been added as dependent claims from claims 32 and 35, respectively. The new claims are limited to methods in which the test compounds inhibit the activity of PKC epsilon and provide a reduced level of anxiety. The new claims are supported in the specification at page 7, lines 18-20, inter alia, and are analogous with claim 29 which depends from claim 10. No new matter is added by any of these amendments and their entry is respectfully requested.

Response to Rejections

Claims 10 and 29, 31-33 and 35-39 were rejected under 35 U.S.C. §112, first paragraph, as not enabled for a variety of reasons discussed below. This rejection is respectfully traversed.

The Examiner makes his rejection on two general bases: 1) that the specification fails to provide sufficient evidence that a compound that affects PKCε would have anxiety modulating activity and 2) that the specification fails to provide sufficient guidance as to what would have been considered a subject or animal model of anxiety such that a skilled artisan would have had to use undue experimentation to make and use the invention (see Office Action, Paper No. 18, at page 7, lines 19-24). A number of issues are raised by the Examiner under each of these areas. Applicant will address the second basis for rejection first.

With regard to a subject or animal model for anxiety, Applicant maintains that, from the teachings in the specification, one of ordinary skill in the art would readily understand what animal models could be used in the practice of the claimed invention. The specification teaches on page 32, at lines 8-12, that the ability of test compounds to inhibit, enhance or modulate the function of PKCε will be measured in suitable animal models, for example, mouse models for monitoring the compound's ability to inhibit or reduce anxiety. Animal models for monitoring anxiety are well known in the art. As

evidence of this, Applicant encloses two recent publications that review animal models for the study of anxiety. The first publication is a chapter from a book entitled *Current Protocols in Neuroscience* (Taylor, G.P. Ed., (2000) Vol. 2 John Wiley & Sons, Inc., attached as Exhibit A). In a chapter entitled "Animal Tests of Anxiety" (Id. at page 8.3.1), protocols for several of the most commonly used animal tests of anxiety are described, including the elevated plus maze test (at page 8.3.6), which is among the animal tests used by the present inventors. The first sentence in the chapter states that animal tests of anxiety are used to screen novel compounds for anxiolytic or anxiogenic activity, *inter alia*. The second publication is a chapter from a book entitled *What's Wrong With My Mouse ?* (Crawley, J.N., (2000) Wiley-Liss, attached as Exhibit B). In a chapter entitled "Emotional Behaviors: Animal Models of Psychiatric Diseases" (Id. at page 179), the author presents a list of dozens of animal models of anxiety-related behaviors (see Table 10.1 at page 182). Among these are listed both the open-field test and the elevated plus maze tests used by the present inventors. Thus, it is clear that there are numerous animal models of anxiety that are well known in the art for use in the same sort of screening for anxiolytic and anxiogenic compounds as described in the specification. One of ordinary skill in the art would have had no difficulty in choosing a suitable animal model from among those that are well known and well characterized in the art.

With regard to some of the specific issues raised by the Examiner with respect to the animal models, the Examiner asks (Paper No. 18, at page 3, lines 15-22) what would be considered a subject that could be used for determining the efficacy of a compound in modulating the state of anxiety, could any animal be a subject, could an animal that had more than one characteristic be used and if any of the listed symptoms were present could the animal still be an appropriate subject and how would the artisan know that the change in behavior is due to anxiety and not some other cause.

Applicant believes that all of these concerns raised by the Examiner are answered simply by the fact that, as explained above, there are many recognized animal models of anxiety. These animal models define the particular animals that are suitable for use in the particular models and the types of behaviors that are indicative of an

increase or decrease in anxiety levels in the test animals. Thus, one of ordinary skill in the art need only choose from among the well-characterized tests to answer the questions that the Examiner has raised. As an example, Applicant directs the Examiner's attention to the publication cited above entitled *Current Protocols in Neuroscience.* In describing the protocol for the elevated plus-maze on page 8.3.6, the authors indicate that the test can be used with male or female rats or mice, referring the reader to the "Critical Parameters" section. Under the "Critical Parameters" section (at page 8.3.15), the authors explain that this particular test has been validated for male rats and male mice and also is reliable for female rats. No mention is made of female mice. One of ordinary skill in the art would therefore have understood that male rats, male mice and female rats would be appropriate as test animals for this test but that female mice may not provide an appropriate test animal for use in this particular test. Under the "Anticipated Results" section (page 8.3.17), the authors describe the behavioral results expected for anxiolytic drugs and for anxiogenic drugs, including an increase in the percentage of time spent in the open and in the percentage of entries onto the open arms in response to anxiolytic compounds and a decrease in these measures for anxiogenic treatments. Similar information is available in the art for other standard animal models of anxiety.

The Examiner questions whether the female mutant mice have reduced anxiety-related characteristics and how results obtained in the animals are indicative of an anxiety animal model in view of the marked differences in the behavior phenotype of six inbred strains of mice.

As the Examiner has recognized, the PKC$\varepsilon^{-/-}$ mice described in the specification are not intended to be used as the test animals in the claimed methods for identifying modulators of PKCε as these mutant mice do not express PKCε. This would certainly be apparent to one of ordinary skill in the art. As explained in the previous response, the PKC$\varepsilon^{-/-}$ mice described in the specification were used to prove the link between PKCε and anxiety by demonstrating that mice that do not express PKCε exhibit lower anxiety levels in standard animal tests of anxiety. In two of three standard animal anxiety tests described in the specification, the open-field test and the exploration of a novel object test, no statistical difference is seen between the male and female mutant

mice in the level of anxiety exhibited. The data for the open-field test is shown in the specification at page 55, line 16-18 and Figures 7 and 8. The gender data for the exploration of a novel object test is not shown in the specification but no gender differences were seen. In the elevated plus-maze test, there was a difference between male and female mutant mice but this is not unexpected since the elevated plus-maze test is well known to yield uncertain results with female test animals, as explained in the Declaration of Robert Messing submitted with the previous response.

With regard to the issue relating to the marked differences in the behavior phenotype of six inbred strains of mice (by which Applicant understands that the Examiner is referring to the Abstract by Rogers et al. that was referred to in the previous Office Action), Applicant does not doubt that different inbred mouse strains may exhibit different behavioral phenotypes. However, Applicant does not understand the Examiner's concern in this regard with respect to the test animal model. Any difference in behavioral phenotype exhibited by different inbred strains is eliminated by using the same animal strain for anxiety-testing in the presence and the absence of the compound to be tested, as is commonly done in a well-controlled experiment, as one of ordinary skill in the art would undoubtedly be aware.

Applicant now addresses the first basis of the rejection, that is, the issue of whether a compound that affects PKCε would have anxiety-modulating activity. Applicant has demonstrated, for the first time, a relationship between PKCε and anxiety. The Examiner admits that Applicant's results show that PKCε null mutation causes decreased anxiety in mutant mice (see Paper 18, page 5, lines 23-25). To further demonstrate the link between PKCε activity and anxiety, Applicant has shown that $GABA_A$ receptors of the $PKC\varepsilon^{-/-}$ mice are more sensitive to benzodiazepines (specification, Example 7, page 60-62). $GABA_A$ agonists are well known as anxiolytics. Benzodiazepines, in particular, are a class of anxiety-reducing drugs that bind with high affinity to $GABA_A$ receptors in the central nervous system, and increase the Cl^- channel open time (specification at page 5, lines 12-15). Muscimol binds competitively to $GABA_A$ receptors and can elevate Cl^- conductance independently of endogenous GABA (specification at page 5, lines 18-19). Flunitrazepam, a benzodiazepine, enhanced the

muscimol-stimulated Cl^- uptake two-fold in microsacs from PKCε$^{-/-}$ mice (specification, Fig. 15B) compared to that seen in microsacs from wild type mice. These findings demonstrate that $GABA_A$ receptors in PKCε$^{-/-}$ mice are more sensitive to allosteric modulation by benzodiazepines (specification, page 16, lines 14-20). To demonstrate that the enhanced sensitivity to allosteric modulators of $GABA_A$ receptors of PKCε$^{-/-}$ mice is the result of lack of PKCε, muscimol-stimulated Cl^- uptake was also examined in *wild-type* microsacs that had been treated with a selective inhibitor of PKCε, εV1-2 (specification, Figure 15C). The εV1-2-treated wild type microsacs also showed enhanced muscimol-stimulated Cl^- uptake in the presence of flunitrazepam. Thus, the loss of PKCε activity either as a result of genetic deletion (as in the PKCε$^{-/-}$ mice) or through selective pharmacological inhibition (as in the wild type inhibited with εV1-2) leads to a similar effect on muscimol-stimulated Cl^- uptake. These results strongly support the conclusion that absence of PKCε-mediated signaling in adult neurons is responsible for enhanced sensitivity of $GABA_A$ receptors to allosteric modulators in PKCε mutant mice. In addition, no alterations in levels of PKC isozymes other than PKCε were observed in PKCε$^{-/-}$ mice (Application, Fig. 15D) suggesting that the responses to $GABA_A$ agonists observed in PKCε $^{-/-}$ mice appear to be due to the loss of PKCε rather than an increase of another PKC isoform. Therefore, Applicant respectfully submits that these results clearly establish the link between PKCε activity and anxiety and makes it reasonable to expect that compounds that modulate the activity of PKCε will have an effect on anxiety. The Examiner has not presented any evidence why this would not be the case.

The Examiner argues that a compound that inhibits PKCε in a purified preparation may inhibit other enzymes or other isoforms of PKC, and suggests that, for example, if broad kinase inhibitor, that inhibited different kinases, affected anxiety due to its nonspecific inhibitory activity, such an effect would not be specific for PKCε or even PKC. However, the fact that a compound may have an effect on targets other than PKCε is irrelevant. Applicant is not claiming a method of identifying selective inhibitors of PKCε and a compound need not be a *selective* modulator of PKCε in order to be useful in

the claimed methods. Many useful drugs are not selective for their desired target. One of ordinary skill in the pharmaceutical arts will understand that specificity for the target is only one factor that needs to be assessed along with many others such as potency, toxicity, bioavailability, etc., in the development of the ultimate therapeutic compound. The present invention provides a useful screening method for narrowing the field of possible compounds to be assessed in an animal model of anxiety by providing a molecular target (i.e., PKCε), whose activity in the presence and absence of large numbers of test compounds can be readily determined.

Regarding the issue raised on page 4, first full paragraph of the Office Action, of the method of testing the PKCε modulatory activities of a compound and whether the in vitro obtained results can be extrapolated to an in vivo system, Applicant believes that this particular issue is relevant only to claims 32 and 33. As all other pending claims (that is, claims 10, 29, 31 and 35-39) have an explicit step of determining the effect of the test compound in an animal model, there is no need to "extrapolate" the *in vitro* results to the *in vivo* situation. Nevertheless, Applicant maintains that the link between PKCε and anxiety has been sufficiently demonstrated by the present invention such that modulators of PKCε activity would reasonably be expected to have anxiety-modulating activity.

The Examiner further suggests that there is no evidence that the changes seen in GABA receptor function, as measured by chloride transport, is specific to PKCε-mediated changes in anxiety because a compound that inhibits only PKCε in a purified preparation may inhibit other enzymes or isoforms of PKC. The Examiner goes on to ask how inhibition of a partially purified enzyme preparation or a cell lysate by a compound can be relied upon for anxiety modulatory activity of the compound.

The Examiner appears to be asking several different questions here. With respect to $GABA_A$ receptor function and PKCε, Applicant directs the Examiner's attention to the specification at Example 7, page 61, line 21 through page 62, line 11. Applicant has shown that the enhanced sensitivity to allosteric modulators of GABA receptors seen in the $PKC\varepsilon^{-/-}$ mice is due to the loss of PKCε activity because enhanced sensitivity is also seen in wild-type microsacs that had been treated with a *selective* PKCε

inhibitor, εV1-2. That is, whether the PKCε function is impaired by genetic deletion (as in the mutant mice) or by pharmacological inhibition (as in the wild-type PKCε inhibited by εV1-2) a similar enhanced sensitivity to allosteric modulators of GABA receptors is observed, as shown by enhanced muscimol-stimulated Cl^- uptake. If the Examiner is suggesting that a compound would have to be a selective inhibitor of PKCε in order to modulate anxiety, Applicant strongly disagrees with such contention as discussed above.

With respect to the issue of the use of a partially purified enzyme preparation or cell lysate to determine the inhibition of PKCε and whether such inhibition can be relied upon for anxiety-modulatory activity *in vivo*, Applicant assumes that the Examiner's concern relates to the specificity of the inhibition measured in such a milieu. The Examiner appears to be concerned that compounds that inhibit other activities, in addition to the PKCε activity, could be selected in such an assay. Applicant maintains that a test compound need not *selectively* inhibit (or enhance) PKCε activity in order to be useful as an anxiety-modulator. A compound need only non-selectively modulate PKCε activity to be useful. Selective PKCε inhibitors (and enhancers) may be preferable but they are not required. Furthermore, partially purified enzyme preparations and cell lysates are routinely used to identify compounds having a selective effect on the activity of a particular enzyme by using the appropriate controls, as would be readily apparent to one of ordinary skill in the art. Moreover, for the methods of claims 10, 29, 31 and 35-39, it is unnecessary to rely on the PKCε-modulatory activity alone as the ability of the test compound to modulate anxiety is directly tested in a animal model after the compound is determined to have some level of PKCε-modulatory activity.

Finally, the Examiner asks whether complete elimination of PKCε activity, similar to that in the knock-out mouse, can be achieved using a compound and the Examiner suggests that there is no guidance in the specification as to what level of inhibition of PKCε would cause decreased anxiety in a test animal.

The present invention provides useful screening assays using a newly-identified molecular target for identifying compounds that modulate anxiety. Prior to the work of the present inventors, it was not known that PKCε was associated with anxiety. The

inventors have shown that elimination of PKCε, in a knockout mouse, results in animals with reduced anxiety levels as compared to PKCε normal animals. It is reasonable to expect that a compound need not completely eliminate the activity of PKCε in order to exhibit some effect on anxiety level. One of ordinary skill in the art would have no difficulty in determining the level of PKCε inhibition necessary to provide a useful level of anxiolytic activity. Such a determination is routine and well within the competence of an ordinary worker in the field. The specification does not have to provide guidance for what is well known and routine.

For the reasons described in the preceding sections, the Applicant respectfully requests withdrawal of the rejection of claims 10 and 29, 31-33 and 35-39 under 35 U.S.C. §112, first paragraph.

I. DEFINITENESS

The Examiner has rejected claims 10, 29, 31, and 35-39 under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

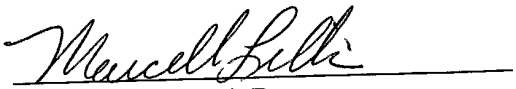
The Examiner states that claims 10 is vague and indefinite because it is unclear what is meant by the phrase "a test animal subject to anxiety". The Examiner states that claims 35 is vague and indefinite because it is unclear as to what is meant by the phrase "an animal subject to anxiety". Claims 10 and 35 have been amended to recite that the test compound is administered to a test animal and the phrase "subject to anxiety" has been deleted. As explained in an earlier section under the response to the rejection under 35 USC 112, first paragraph, one of ordinary skill in the art would well understand what a suitable "test animal" would be.

Accordingly, in view of the above remarks, it is submitted that this application is now ready for allowance. Early notice to this effect is solicited.

If in the opinion of the Examiner, a telephone conference would expedite the prosecution of the subject application, the Examiner is invited to call the undersigned at (650) 843-5604.

Cooley Godward LLP
Attn: Patent Group
Five Palo Alto Square
3000 El Camino Real
Palo Alto, CA 94306-2155
Tel: (650) 843-5000
Fax: (650) 857-0663

Respectfully submitted,
COOLEY GODWARD LLP

By: 
Marcella Lillis, Ph.D.
Reg. No. 36,583

VERSION WITH MARKINGS TO SHOW CHANGES

10. (Twice Amended) A method of identifying a compound that modulates anxiety, said method comprising:
selecting, as a test compound, a compound that modulates the activity of PKCε,
[and]
administering said test compound to a test animal [subject to anxiety to determine], and determining whether [said] anxiety is modulated in said test animal.

29. (Twice Amended) The method of claim 10, wherein said test compound selectively inhibits the activity of PKC epsilon and said anxiety is reduced.

31. (Twice Amended) The method of claim 29, wherein said reduction of anxiety in a test animal is determined by a reduction in one or more symptom of anxiety selected from the group consisting of: decreased locomotor activity, decreased time in open areas, decreased exploratory behavior, and increased basal level of a stress hormone.

32. (Amended) A method of identifying compounds that modulate anxiety, said method comprising:
measuring the activity of PKC epsilon in the presence and absence of a test compound, wherein said measuring is performed in other than an animal, and
[exposing a functional PKC epsilon to a test compound,]
determining whether the test compound modulates the activity of PKC epsilon, wherein test compounds that modulate the activity of PKC epsilon are identified as compounds for modulating anxiety.

33.(Amended) The method of claim 32, wherein said [exposing] measuring is performed in [a] an isolated cell or cell lysate.

35. (Twice Amended) A method of identifying compounds that modulate anxiety, said method comprising:

measuring the activity of PKC epsilon in the presence and absence of a test compound, wherein said measuring is performed in other than an animal;

[determining whether]selecting a [the] test compound that modulates the activity of PKC epsilon[,];

administering to [an] a test animal [subject to anxiety a] the selected test compound that modulates the activity of PKC epsilon; and

determining whether the test animal's anxiety is modulated, wherein test compounds that modulate the animal's anxiety are identified as compounds for modulating anxiety.

36. (Amended) The method of claim 35, wherein said measuring is performed in [a] an isolated cell or cell lysate.

37. (Reiterated) The method of claim 35, wherein said measuring is performed in an in vitro assay.

38. (Amended) The method of claim 35, wherein said administering occurs under conditions in which the test animal, in the absence of the test compound, displays symptoms of anxiety.

39. (Amended) The method of claim 35, wherein said [second] determining step is performed after the animal is exposed to an anxiety-producing stimulus.

54. (New) The method of claim 32, wherein said test compound selectively inhibits the activity of PKC epsilon and said anxiety is reduced.

55. (New) The method of claim 35, wherein said test compound selectively inhibits the activity of PKC epsilon and said anxiety is reduced.